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RESEARCH ARTICLE

The effects of ultraviolet B beams on programmed cell death activities in *Staphylococcus epidermidis*

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ABSTRACT

Objectives: Bacterial skin diseases caused by drug-resistant *Staphylococcus epidermidis* are known as a big problem in the field of treating management of infectious diseases. Progression of resistant strains has led to use phototherapy in parallel with pharmacotherapy. In this short survey, we tried to obtain a logic Ultraviolet Radiation protocol to induce the process of programmed cell death in irradiated *Staphylococcus epidermidis*.

Methods: The samples of *Staphylococcus epidermidis* were classified in 4 categories. Each plate which is known as a category included well-grown colonies of bacteria. One plate was taken as a control sample and the left three plates were irradiated by UVB (302 nm) in 10 minutes from the distance of 8 cm. The irradiated plates were kept in a dark cell and for 1, 24 and 72 hours respectively. Then, total genomic DNA molecules pertaining to all of the colonies comprising control and irradiated samples were harvested by DNP kit and the extracted DNA molecules were running upon the 1% agarose gel together with ethidium bromide.

Results: Control and irradiated samples were studied for probable changes in their macroscopic, microscopic characteristics and then the DNA pattern relating to each group was detected for any variation including smear or DNA laddering bands. No changes were observed in different bacterial properties. No apoptosis were observed in irradiated samples.

Conclusion: UVB is a strong apoptosis stimulus in organisms. However, authors of the present study strongly reject the apoptotic effect of UVB irradiation in the format of the present protocol. *J Microbiol Infect Dis 2015;5(1): 21-24*

Key words: *In vitro*, programmed cell death, *Staphylococcus epidermidis*, UVB

UVB ışınlarının Stafilokok epidermidisdeki programlanmış hücre ölüm faaliyetleri üzerine etkileri

ÖZET

Amaç: İlaca dirençli *Staphylococcus epidermidis* neden olduğu bakteriyel deri hastalıkları, enfeksiyon hastalıklarının tedavi alanında büyük bir problem olarak bilinmektedir. Dirençli suşların artması farmakoterapiyle beraber fototerapinin de kullanılmasına yol açmaktadır. Bu kısa araştırmada, ışına maruz kalmış *Staphylococcus epidermidis*in programlanmış hücre ölüm sürecini başlatacak anlamlı bir Ultraviole Radyasyon protokolünü oluşturmaya için uğraştık.

Yöntemler: *Staphylococcus epidermidis* örnekleri 4 kategoride sınıflandırılmıştır. Bakteri kolonilerinin çoğalma durumuna göre her plak bir kategoriye dahil edildi. Bir plak kontrol olarak ve diğer 3 plağa ise 8cm uzaktan 10 dakika süreyle ultraviolet B (UVB) (302 nm) ışını uygulandı. Işına maruz kalan plaklar sırayla 1saat, 24 saat ve 72 saat süreyle karanlık bir hücre tutuldu. Daha sonra, kontrol ve ışınlanmış örnekler içeren tüm kolonilerin toplam genomik DNA molekül parçaları DNP kiti ile çoğaltıldı ve ekstre edilmiş DNA molekülleri, etidyum bromür ile birlikte % 1 agaroz jel üzerinde yüklenip yürütüldü.

Bulgular: Kontrol ve ışınlanmış örneklerde makroskopik, mikroskopik özelliklerindeki olası değişiklikler için çalışma yapıldı ve sonra her gruba ilişkin DNA merdiven bantları veya yaymalarını da içeren DNA örnekleri üzerinde herhangi bir değişiklik olup olmadığı belirlendi. Farklı bakteriyel özelliklerde herhangi bir değişiklik gözlenmemiştir. Işınlanmış örneklerde hiçbir apoptoz gözlenmemiştir.

Sonuç: UVB organizmalarda güçlü bir apoptoz uyarıcıdır Ancak, bu çalışmanın yazarları bu protokol formatında UVB ışınlamasının apoptotik etkisinin olmadığını şiddetle reddetmektedir.

Anahtar kelimeler: in vitro, programlanmış hücre ölümü, *Staphylococcus epidermidis*, UVB

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INTRODUCTION

The human skin encompasses a vast diversity of microbial normal flora, including Coagulase-Negative Staphylococci (CNS). CNS comprises the gram positive bacterium of *Staphylococcus epidermidis* as the most common colonizer of the human body which involves armpits, inner surfaces of nares. The predominant strains of *S.epidermidis* support health of the skin against pathogenic bacteria like *Staphylococcus aureus*. However, some opportunistic pathogenic strains of *S.epidermidis* are recognized as device-related bacterial infectious agents. Besides, a wave of Methicillin-Resistant *S.epidermidis* (MRSE) has been distinguished among nosocomial infectious isolates.[1-6]

Today, the multi-drug-resistant strains are significantly increasing around the world. Thus, the use of UV-phototherapy may be a good choice for replacing or administrating in parallel with chemotherapy.[2,7]

The use of UV beams may help to induce the programmed cell death (apoptosis) in irradiated bacterial cells. For this purpose, the agarose gel electrophoresis seems a suitable tool for observing apoptosis feature in the format of DNA laddering bands. The appearance of apoptosis may lead to use the protocol as an *in vivo* treating management for different microbial organism.[4,8-14]

The aim of this survey is to detect programmed cell death (apoptosis) activities in the irradiated bacterial cells of *S.epidermidis* via a determined UVB-irradiation protocol designed by the authors.

METHODS

The bacterial samples of *S.epidermidis* were provided from the microbial resources of the microbiology laboratory, Islamic Azad University, Shahr-e-Qods branch. The accuracy of samples was confirmed by the routine practical assays, including Gram staining, microscopy and biochemical tests.[3]

Same amounts of the bacteria were inoculated into four plates of nutrient agar (Merck KGaA, Darmstadt, Germany) and incubated at 37ºC for 3 days to appear well-grown colonies.

One plate presumed as control sample and the other three plates were irradiated by UVB light of 302 nm with a distance of 8 cm away from UV-transilluminator (Upland, CA, U.S.A.) for 10 minutes. Simultaneously, the quantity of UV beams and heat was respectively estimated in maximum and minimum volumes. According to our protocol, the three irradiated plates were maintained in a dark cell for respectively 1, 24 and 72 hours while the control plate did not undergo UVB-exposure. In the next step, the total genomic DNA of the four samples, including control and irradiated colonies were extracted by DNP kit through following company's protocol (50T, CinnaGen Inc.).[4,9,10]

In the following step, the harvested DNA molecules relating to each studied group were running on agarose gel electrophoresis comprising ethidium bromide 1%. Furthermore, a DNA weight marker III produced by CinnaGen Company was applied to compare and analyze the created bands. The density of RNA molecules was ignorable and not significant. In the final stage, the created DNA molecules bands belonging to control and UVB-exposed *S.epidermidis* bacterial cells were assessed for probable DNA laddering bands as a symbol pattern of programmed cell death (Figure 1).[4,8-16]

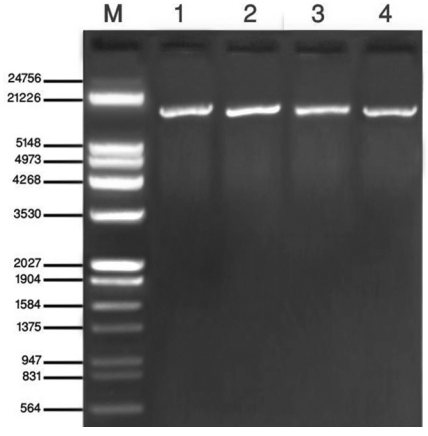

Figure 1. The running of DNA molecules relating to control and UVB- irradiated colonies of *S.epidermidis*, on 1% agarose gel.

Lane M: weight marker III (CinnaGen Company) indicating the situation of DNA bands around 18 kbp.
Lane 1: DNA band of control sample.
Lane 2: DNA band of 10-minute-irradiated sample, one hour incubation within a dark cell after UV radiation.
Lane 3: DNA band of 10-minute-irradiated sample, twenty four hours incubation within a dark cell after UV radiation.
Lane 4: DNA band of 10-minute-irradiated sample, seventy two hours incubation within a dark cell after UV radiation.

RESULTS

A complete evaluation of macroscopic and microscopic properties pertaining to control and UVB-irradiated bacterial colonies of *S.epidermidis* was achieved for detecting presumable variations. The

outcome of observations devoted neither macroscopic nor microscopic differences in control and UVB-exposed colonies.

Also, the control and UVB-irradiated samples were investigated from molecular characterisitics. No dissimilarity and alternation including smear, DNA laddering bands or any deformities was recognized in DNA patterns pertaining to 10-minute-UVB-exposed and control samples (Figure 1). The practice was repeated two times.

DISCUSSION

The high rating of resistance to different antibiotics such as aminoglycosides, macrolides, methicillin, tetracyclines and vancomycin among *S.epidermidis* strains isolated from patients with nosocomial infections encourages physicians and clinicians to use the strategy of therapeutic photomedicine approaches for treating skin infectious diseases caused by drug-resistant strains of *S.epidermidis*.[5,17-20]

Ultraviolet or the beam of beyond violet is a limited spectrum (200-400 nm) including UVC, UVB and UVA which is located between visible and X-ray wavelengths. UV radiation (UVR) has apoptotic, carcinogenic and mutagenic (DNA damage) side effects in both eukaryotic and prokaryotic cells. However, a beneficial reaction of vitamin D synthesis is detected when the epidermis layer of the human skin is exposed to UVB light.[4,9-14,21-22]

The apoptosis triggering effect of UVB on irradiated bacterial cells is detectable via running the harvseted total genomic DNA molecules on 1% agarose gel as an classic appropriate microbiological methodology. The apoptotic bacterial DNA molecules show a particular pattern of DNA laddering bands which is completely detectable.[8]

UVB beams are more powerful and much more erythemagenic than UVA rays. However, UVA beams are able to penetrate deeper than UVB rays into the human skin depth. UVC has the least penetration in the human skin texture; thus it is often applied for healing and repairing wounds and scars appeared on the human skin surfaces.[4,21]

UVR and particularly UVB, is able to damage and destruct DNA molecules by producing cyclobutane pyrimidine dimers and free radicals. On the other hand, all the living cells, either prokaryotic or eukaryotic cells have their DNA repair systems.[4,9-14,21,23]

In the parallel with UVB wavelength, the period of irradiation and the distance of UVB lamp is significant. UVB is considered as a bold apoptotic physical agent which may lead to cell death. Previous investigations show that a group of proteases known as caspases have an essential role within the apoptosis process. Among caspases, caspase-3 strongly coordinates the programmed cell death as a part of protective system in which DNA cleavage occurs.[23]

If DNA repair systems and other protective systems fail to accurate UVB side effects, the apoptosis mechanisms start to recover damages. In the present investigation, the protocol of 10-minute UVB irradiation is not able to penetrate the DNA molecules by disabling the bacterial protective systems. It means that, the influence of UVB and the irradiation time are two necessary factors for overcoming the bacterial protective systems. It is important to find a UVR protocol, which treat the bacterial skin infections without any skin cell damages. According to different reports confirming the increase of multidrug resistant bacterial strains, pharmacotherapy has lost its sharp effectiveness. Today, an appropriate, suitable and standard phototherapy is an acceptable and cost effective alternative to replace pharmaceutical treatment.[2,4,23,24]

CONCLUSION

In accordance with aforementioned text, the authors of this article have performed a series of surveys relating to different pathogenic microorganisms within a similar format of UVR protocol. In our previous investigations and the present study, we were not able to trigger apoptosis in different pathogenic microbial agents.[24] It seems that the collection of protective systems belonging to studied microorganisms neutralize UVR effects and the present protocol is not capable with inducing apoptosis within theses cells. For this reason, we persist on the ineffectiveness of the protocol for triggering apoptosis in investigating microorganisms and there is a need for changing the time of radiation.

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